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Synthesis and Biological Evaluation of (6- and 7-Phenyl) Coumarin Derivatives as Selective Nonsteroidal Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 1

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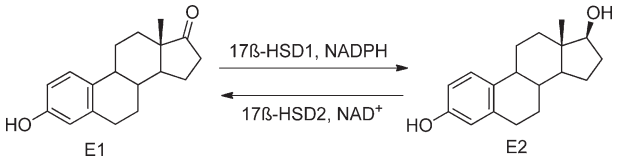
17β-Hydroxysteroid dehydrogenase type 1 (17β-HSD1) is an enzyme that catalyzes NADPH-dependent reduction of the weak estrogen, estrone, into the most potent estrogen, estradiol, which exerts proliferative effects via the estrogen receptors. Overexpression of 17β-HSD1 in estrogen-responsive tissues is related to the development of hormone-dependent diseases, such as breast cancer and endometriosis; thus, 17β-HSD1 represents an attractive target for the development of new therapies. We have discovered that simple coumarines **1** and **2** significantly inhibit 17β-HSD1 in a recombinant enzyme assay, with high selectivity against 17β-HSD2. We postulated that the introduction of various *p*-substituted phenyl moieties to position 6 or 7 of the coumarin core using the Suzuki-Miyaura cross-coupling reaction would provide mimetics of steroidal structures with improved inhibition of 17β-HSD1. The best inhibitor in the series proved to be **6a**, with an IC_{50} of 270 nM, and with exceptional selectivity for 17β-HSD1 over 17β-HSD2 and against the α and β estrogen receptors.

Introduction

Abnormal activities of enzymes that are involved in the local biosynthesis of steroid hormones can significantly contribute to the development of many hormone-related diseases. Hormone-dependent breast cancer and prostate cancer are the most common malignancies in western women and men, respectively.[1,2] The incidence of hormone-dependent cancers is continuously rising, and they still represent one of the major causes of death.

Among the enzymes that act at the final stages of biosynthesis of the steroid sex hormones, the 17β-hydroxysteroid dehydrogenases (17β-HSDs) have important roles through their catalysis of redox reactions at position 17 of the steroidal core, thus regulating the biological potencies of these hormones.[3,4] The 17β-hydroxy forms of androgens and estrogens have higher affinities toward their corresponding receptors.[3] At present, 14 mammalian 17β-HSDs have been identified.[4] With the exception of 17β-HSD type 5 (17β-HSD5[1]), which is a member of the aldo-keto reductase superfamily, all of the 17β-HSDs belong to the short-chain alcohol dehydrogenase reductase superfamily, although their overall homologies are low.[5] The activities of the 17β-HSDs are related to the pathogenesis of a number of diseases,[4] and therefore, they represent important targets for the design of new therapies. Their roles in the control of cell proliferation are particularly important for the development and progression of hormone-dependent breast and prostate cancers.[6−8] However, only 17β-HSD types 1, 2, and 3 appear to have roles solely in steroid metabolism,[4,9] while the other 17β-HSDs share substrates with enzymes involved in other metabolic pathways, e.g., lipid metabolism.[10] The most extensively characterized of the 17β-HSDs is 17β-HSD1,[11−15] which catalyzes NADPH-dependent reduction of the weakly active estrone (E1) into the potent estrogen estradiol (E2) (Chart 1).[9] In this manner, 17β-HSD1 can regulate the occupancy of the estrogen receptors (ERs) and thus the proliferative effects of the estrogens.[16,17]

Chart 1. Reactions Catalyzed by 17β-HSD1 and 17β-HSD2



17β-HSD1 is expressed mainly[18] in ovaries,[19] placenta,[20] and breast tissue,[21] and also in ectopic endometrium.[22] Thus, it is related to the development and progression of hormone-dependent breast cancer,[8] as well as endometriosis.[22] E2 stimulates the growth of breast cancers,[23] and the intratumoral E2/E1 ratio in ER-positive breast cancer tumors that depends on 17β-HSD1 activity is significantly higher in postmenopausal patients than in premenopausal patients.[24] 17β-HSD1 expression has also been reported for more aggressive hormone-dependent breast cancers that show poor prognosis and shorter remission times of the disease.[8,25] The incidence of breast cancer is higher in postmenopausal patients, where the production of estrogens is limited to only peripheral tissues. There, the estrogens are formed and act on the same

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[1] Abbreviations: 17β-HSD1, 17β-hydroxysteroid dehydrogenase Type 1; 17β-HSD2, 17β-hydroxysteroid dehydrogenase Type 2; 17β-HSD5, 17β-hydroxysteroid dehydrogenase Type 5; E1, estrone; E2, estradiol; ER, estrogen receptor; NAD(H), nicotinamide dinucleotide; NADP(H), nicotinamide dinucleotide phosphate; RBA, relative binding affinity; SM, Suzuki-Miyaura.

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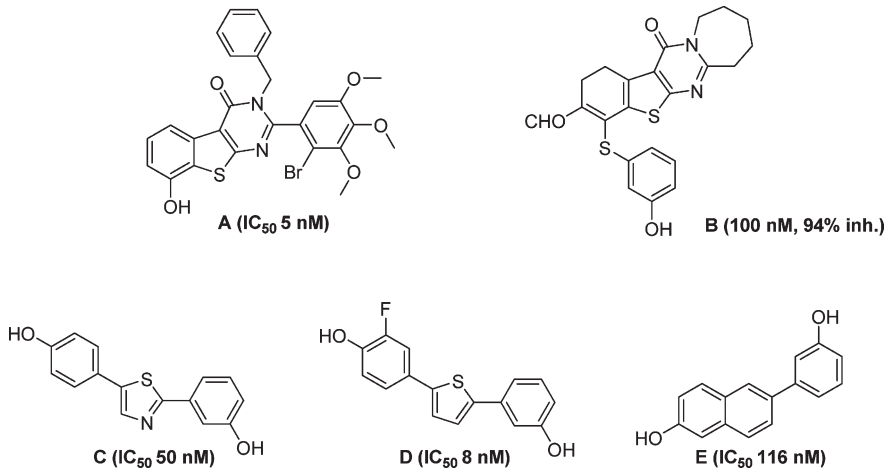
Chart 2. Reported Structures of the Most Potent Nonsteroidal Inhibitors of 17β-HSD1



(intracrine action) and neighboring (paracrine action) cells.[26,27] The intracrine concept for lowering the concentrations of active hormones in target cells has already been successfully applied for the treatment of prostatic diseases by the use of 5α-reductase inhibitors. Steroidal 5α-reductase inhibitors, e.g., finasteride and dutasteride, have been drugs of choice for the treatment of benign prostatic hyperplasia. A great effort was also put in the development of nonsteroidal 5α-reductase inhibitors, which would exert fewer side effects than steroidal inhibitors.[28−30]

The selective inhibition of the local 17β-HSD1 activity would thus provide specific therapies that would be beneficial and supplemental to current treatments with selective ER modulators and aromatase inhibitors.

Although clear structure−activity relationships have been established for 17β-HSD1, no successful clinical drug candidates have been designed yet.[31] To be suitable as a potential drug candidate, 17β-HSD1 inhibitors must meet two requirements:[32] they should lack affinity toward ERs, to avoid estrogenic effects; and they should show significant selectivity for 17β-HSD1 over 17β-HSD2, the latter of which has a protective role by catalyzing the opposite reaction in estrogen responsive tissues (NAD^+-dependent oxidation of E2 to E1).[33−35]

Many potent steroidal inhibitors of 17β-HSD1 that show nanomolar IC_{50} values have been reported and extensively reviewed.[5,36−38] However, the majority of these steroidal inhibitors are not selective enough for 17β-HSD1, and they also have estrogenic properties.[39] There is thus great interest in the identification of new nonsteroidal and selective 17β-HSD1 inhibitors.

Recently, several groups have reported selective nonsteroidal inhibitors of 17β-HSD1 with nanomolar or low micromolar IC_{50} values that have been determined in enzyme-based or cell-based assays. Most of these are lipophilic, rigid, aromatic molecules that occupy the steroid substrate-binding region in the active site of 17β-HSD1 (Chart 2). Of note here, the inhibitory potencies of different inhibitors reported by various research groups are difficult to compare, since different assay conditions are often used, especially regarding the various enzyme sources (purified enzymes, transfected or intact cells containing the enzyme), the various substrate concentrations, and the various cofactor species. Therefore, for more comparable results, it is recommended to use an inhibitor that has been previously characterized by at least one other research group as a positive control in any inhibition assays.

Inhibitors of 17β-HSD1 that are based on a benzothienopyrimidinone and dihydrobenzothiophene core (Chart 2 A,B, respectively) were discovered in lead-finding processes based on the structures of known natural inhibitors, like coumestrol and kaempferide. Compounds A[40] and B[41] of Chart 2 showed remarkable inhibitory potencies for 17β-HSD1, at very low nanomolar concentrations. They also lacked estrogenic effects, and in addition, compound A reduced tumor growth in vivo. However, their selectivities for 17β-HSD1 must be improved.[40,41] Bis(hydroxyphenyl) substituted azoles and thiophenes showed potent 17β-HSD1 inhibitory activities with 50 nM and 8 nM IC_{50} values for C[42] and D[43] of Chart 2, respectively. These were selective against 17β-HSD2 (selectivity factor of nearly 100) and had negligible affinities for the ERs, with no stimulation of cell proliferation in the T-47D cell line. Furthermore, they reduced estrogen-responsive cell growth of T-47D cells[44] and showed excellent pharmacokinetic parameters.[43] The principle feature of these compounds is the fixed distance of approximately 12 Å between the two hydroxyl groups on both of the phenyl rings, which resembles the distance between the two oxygens at positions 3 and 17 of E2.

(Hydroxyphenyl)naphthalenes (e.g., Chart 2 E)[45] strongly inhibited 17β-HSD1 with nanomolar IC_{50} values and good selectivities against 17β-HSD2 and the ERs. These compounds act as steroidal mimetics, with a flat lipophilic bicyclic core that is substituted with two polar groups in the appropriate positions, to resemble the steroid O(3) to O(17) distance. These structures allow polar interactions with the His221/Glu282 and Ser142/Tyr155 that are located at the opposite ends of the mostly hydrophobic substrate-binding cleft of 17β-HSD1.[45,46] Recently, a combination of ligand- and structure-based design resulted in heterocyclic substituted biphenylols and their aza-analogues as new 17β-HSD1 inhibitors.[47]

Here, we describe the design, chemical synthesis, and 17β-HSD1 inhibitory activities of new coumarin derivatives. We discuss their selectivities against 17β-HSD2, ERα, and ERβ, and explain the biological data with reference to their inhibition of 17β-HSD1 through computer docking studies.

Results and Discussion

Design of new 17β-HSD1 Inhibitors with the Coumarin Core. We postulated that a coumarin can be used as a planar bicyclic core structure allowing for the introduction of

different substituents to obtain steroidal mimetics with potential for selective 17β-HSD1 inhibition. A lactone group with two proton acceptor oxygens lead to additional favorable interactions in the active site. Although the coumarin core is a part of many natural and synthetic compounds with wide pharmacological activity,[48] coumarin structures that inhibit 17β-HSD1 are rare. Cinnamic acids, which are natural precursors of the coumarins, were recently reported to be modest inhibitors of 17β-HSD1.[49] Phytoestrogen coumestrol contains the coumarin core, and along with some structurally related flavanoids, these have also been reported as inhibitors of 17β-HSD1.[37,49] Therefore, we tested some simple 3-acetylcoumarin derivatives from our in-house library of compounds for inhibition of 17β-HSD1. This provided us with two 3-acetylcoumarins that had promising 17β-HSD1 inhibitory activity (Chart 3).

Compounds **1** and **2** showed modest inhibition of 17β-HSD1 in the low micromolar range, and they did not act as inhibitors of 17β-HSD2. We hypothesized that either the oxygen of the keto lactone group or the oxygen of the 3-acetyl substituent form similar binding interactions in the active site as those seen for the C17 keto group of E1. To better mimic the steroidal structure and to achieve the correct distance between the steroidal O(3) to O(17) mimicking groups, we introduced various para-substituted phenyl substituents to positions 6 and 7 of this 3-acetylcoumarin scaffold (Chart 4). The calculated distances between the O(3) and O(17) mimicking groups confirmed that these 6-phenyl and 7-phenyl coumarin derivates are mimetics of E1, and they have the potential for inhibition of 17β-HSD1. With this design approach, we simultaneously wanted to avoid the affinity toward ERs, since it is known that phytoestrogens substituted at positions 2 or 3 (e.g., coumestrol, Chart 4) possess estrogenic activity.[50]

Chart 3. Simple 3-Acetylcoumarins **1** and **2** that can Inhibit 17β-HSD1 (the Numbering of the Coumarin Ring System is Shown)

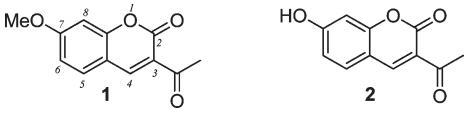
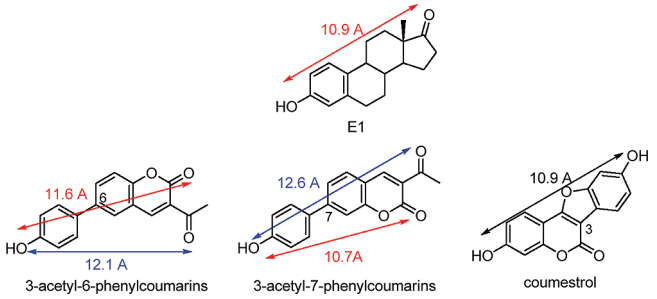


Chart 4. 6-Phenyl and 7-Phenyl Coumarins as Mimetics of E1 and Structurally Related Phytoestrogen Coumestrol



Chemistry

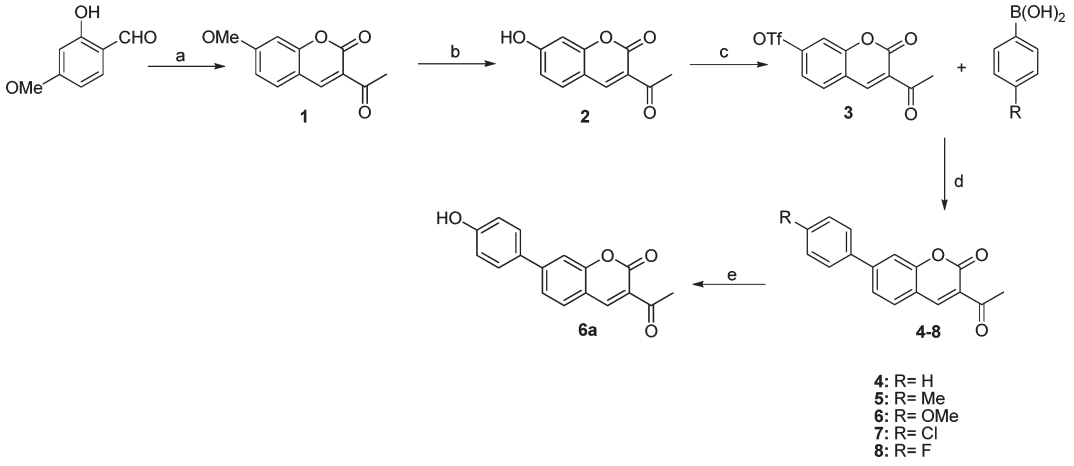
The synthetic preparation of coumarin derivatives **1**−**38** is presented in Schemes 1−4. The key reaction in all of these cases was the Suzuki-Miyaura (SM)[51,52] cross-coupling reaction of various commercially available, *p*-substituted, phenylboronic acids with either 7-triflylcoumarins **3**, **10**, and **17** (Schemes 1, 2, and 3, respectively) or 6-bromocoumarins **22**, **28**, and **34** (Scheme 4).

The synthesis of the 3-acetylcoumarin derivatives **1**−**8** (Scheme 1) started by condensation[53] of commercially available 2-hydroxy-4-methoxybenzaldehyde and ethylacetoacetate, which leads to 3-acetyl-7-methoxy coumarin **1**, which was further demethylated with $AlCl_3$[54] to obtain the 3-acetyl-7-hydroxy coumarin **2** in a satisfactory yield (68%). $AlCl_3$ proved better than BBr_3, since demethylation with BBr_3 gave only a 30% yield. The direct, one-step preparation of **2** from 2,4-dihydroxybenzaldehyde and ethylacetoacetate was hampered by the poor yield of the reaction. Triflation[55] of **2** provided compound **3**, which was used in SM reactions to obtain 7-phenyl cumarins **4**−**8**. The 4′-hydroxyphenyl derivative **6a** was prepared by demethylation of **6** with $AlCl_3$, again in an acceptable yield, which was close to 60%.

Scheme 2 shows the preparation of coumarins **9**−**14**, which started with condensation of commercially available 2,4-dihydroxybenzaldehyde and diethyl malonate,[56] to obtain **9** in an almost 50% yield. After the triflation, compound **10** was subjected to SM reactions with various *p*-substituted phenylboronic acids, to obtain the target 7-phenyl-3-ethoxycarbonyl cumarins **11**−**14**.

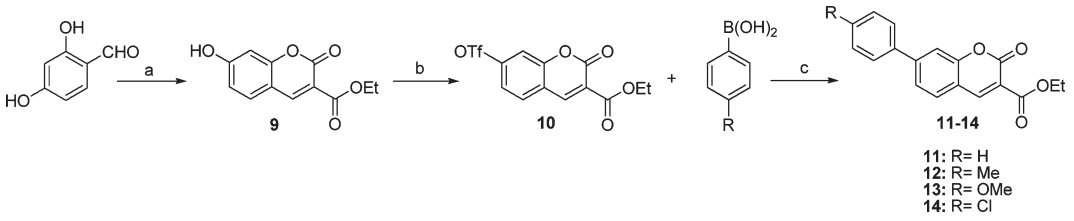
Preparation of coumarins **15**−**21** (Scheme 3) that lack the substitution on position 3 started with the Wittig reaction

Scheme 1. Synthesis of Compounds **1**−**8**[a]



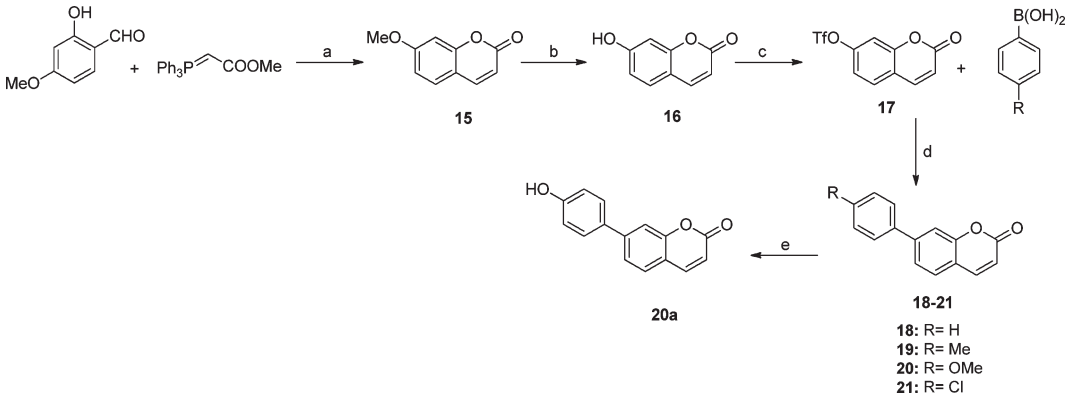
[a] Reagents and conditions: (a) ethyl acetoacetate, piperidine, EtOH, reflux, 4 h; (b) $AlCl_3$, toluene, reflux, 3 h; (c) trifluormethanesulfonic anhydride, pyridine, CH_2Cl_2, 0 °C, 1 h; (d) $Pd(PPh_3)_4$, K_2CO_3, toluene, reflux, overnight; (e) $AlCl_3$, toluene, reflux, 3 h.

Scheme 2. Synthesis of Compounds **9**−**14**[a]



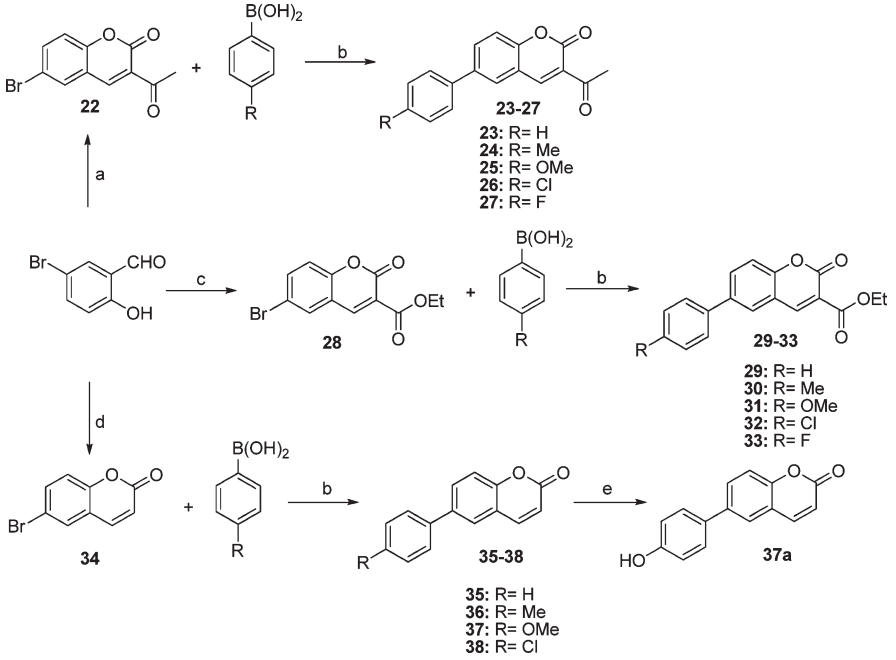
[a] Reagents and conditions: (a) diethyl malonate, piperidine, AcOH, EtOH, reflux, 4 h; (b) trifluormethanesulfonic anhydride, pyridine, CH_2Cl_2, 0 °C, 1 h; (c) $Pd(PPh_3)_4$, K_2CO_3, toluene, reflux, overnight.

Scheme 3. Synthesis of Compounds **15**−**21**[a]



[a] Reagents and conditions: (a) $PhN(Et)_2$, 210 °C, 2 h; (b) $AlCl_3$, toluene, reflux, 3 h; (c) trifluormethanesulfonic anhydride, pyridine, CH_2Cl_2, 0 °C, 1 h; (d) $Pd(PPh_3)_4$, K_2CO_3, toluene, reflux, overnight; (e) $AlCl_3$, toluene, reflux, 3 h.

Scheme 4. Synthesis of Compounds **22**−**38**[a]

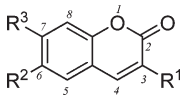


[a] Reagents and conditions: (a) ethylacetoacetate, piperidine, EtOH, reflux, 4 h; (b) $Pd(PPh_3)_4$, K_2CO_3, toluene, reflux, overnight; (c) diethylmalonate, piperidine, AcOH, EtOH, reflux, 4 h; (d) Ac_2O, K_2CO_3, N,N-dimethylformamide, 120 °C, overnight; (e) $AlCl_3$, toluene, reflux, 3 h.

between 2-hydroxy-4-methoxybenzaldehyde and commercially available [(methoxycarbonyl)methylene]triphenylphosphorane.[57] The 7-methoxycoumarin **15** obtained was then demethylated with $AlCl_3$, to obtain 7-hydroxy coumarin **16**; this was further triflated to compound **17**, which was used in SM reactions to obtain the desired 7-phenyl coumarins **18**−**21**. Compound **20a** was obtained by demethylation of **20** with $AlCl_3$. Triflate **3** in general gave poorer yields of SM reactions than **10** and **17**.

Reactions of commercially available 5-bromo-2-hydroxybenzaldehyde with ethylacetoacetate, diethylmalonate, and acetic anhydride provided 6-bromocoumarins **22**, **28**, and **34**,

Table 1. Effects of Nonaromatic Substituents at Positions 3, 6, and 7 of the Coumarin Scaffold on Inhibition of 17β-HSD1 and 17β-HSD2



				inhibition of 17β-HSD1 (%)[a]		inhibition of 17β-HSD2 (%)[a]	
compound	R^1	R^2	R^3	0.6 μM[b]	6 μM	0.6 μM	6 μM
1	-$COCH_3$	-H	-OMe	23	25	n.i.[c]	n.i.
2	-$COCH_3$	-H	-OH	26	57	n.i.	n.i.
3	-$COCH_3$	-H	-OTf	60	100	n.i.	n.i.
9	-COOEt	-H	-OH	n.i.	17	n.d.[d]	n.d.
10	-COOEt	-H	-OTf	n.i.	n.i.	n.d.	n.d.
15	-H	-H	-OMe	n.i.	n.i.	n.d.	n.d.
16	-H	-H	-OH	n.i.	n.i.	n.d.	n.d.
17	-H	-H	-OTf	n.i.	9	n.d.	n.d.
22	-$COCH_3$	-Br	-H	8	19	n.d.	n.d.
28	-COOEt	-Br	-H	n.i.	n.i.	n.d.	n.d.
34	-H	-Br	-H	n.i.	n.i.	n.d.	n.d.
Equilin				81	100	n.d.	n.i.

[a] Recombinant human enzyme overexpressed in *E. coli*, [^{3}H]E1 + E1 [67 nM] and NADPH [100 μM] for 17β-HSD1, [^{3}H]E2 + E2 [300 nM] and NAD^+ [100 μM] for 17β-HSD2; data are mean values of two determinations, with RSD less than 10%. [b] Concentration of inhibitor. [c] No inhibition. [d] Not determined.

respectively. These were then used in SM reactions with various *p*-substituted phenylboronic acids, as shown in Scheme 4. SM reactions with **22** to obtain **23**−**27** proceeded with generally the lowest yields, while SM reactions of **34** to provide compounds **35**−**38** gave the highest yields. Perkin condensation[58] of 5-bromo-2-hydroxybenzaldehyde with acetic anhydride to obtain **34** proceeded with poor yield, and the above-mentioned Wittig reaction with [(methoxycarbonyl) methylene]triphenylphosphorane appeared to be the more convenient choice for improving the yield. Demethylation of **37** with $AlCl_3$ gave **37a**.

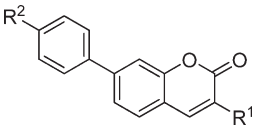
Biological Activity

All of these coumarin derivatives were evaluated for inhibition of recombinant human 17β-HSD1 and recombinant human 17β-HSD2. 3-Acetyl-7-methoxycoumarin **1** was a modest inhibitor of the recombinant 17β-HSD1 enzyme, while its demethylated derivative **2** showed a significant improvement of the inhibitory potency, with 57% inhibition of 17β-HSD1 at 6 μM (Table 1). Further introduction of a large triflic group to position 7 of the coumarin scaffold led to the potent 17β-HSD1 inhibitor **3**, with 60% and 100% inhibition at 0.6 μM and 6 μM, respectively. None of compounds **1**−**3** showed any inhibition of 17β-HSD2 (Table 1), and compound **3** did not inhibit 17β-HSD2 even at 60 μM (data not shown), confirming the high selectivity of this class of inhibitors for 17β-HSD1 over 17β-HSD2. Replacement of the 3-acetyl group with a 3-ethoxycarbonyl substituent (compounds **9** and **10**) led to almost complete loss of inhibitory activity, and the same result was seen with coumarins without substituents at position 3 (compounds **15**−**17**). Introduction of bromine to position 6 of coumarin while keeping position 7 unsubstituted (compounds **22**, **28**, **34**) resulted in very low 17β-HSD1 inhibition (**22**) (Table 1). These results suggested that 3-acetyl coumarin derivatives with large substituents on position 7 can contribute to optimal interactions in the active site of 17β-HSD1, which leads to significant improvements of the inhibitory activity; this almost reached the inhibitory potency of equiline, which was used as a positive control. Equilin, or equine estrogen, is known for its potent inhibition of 17β-HSD1, at submicromolar concentrations.[59]

The triflic group is labile in an aqueous environment, and its reactivity can also lead to unfavorable covalent interactions with other enzymes and receptors, which means that triflates are not desirable as drug candidates. However, the triflic group is one of the most reactive groups in SM cross-coupling reactions with arylboronic and alkylboronic acids, which allowed us to introduce different *p*-substituted phenyl groups to position 7 of the coumarin scaffold. 7-Phenyl coumarins with the 3-acetyl group **4**−**8** (Table 2) showed the most promising inhibition of 17β-HSD1, which was strongly influenced by the nature of the substituent in the para position of the 7-phenyl group. Compounds **5**, **6**, and **6a**, which all have 4′-electron-donor substituents, showed excellent 17β-HSD1 inhibition. Conversely, halogen groups in **7** (Cl) and **8** (F) led to significant reductions in the inhibitory activity. Interestingly, the unsubstituted 7-phenyl group in compound **4** retained strong inhibitory potency, with 57% inhibition of 17β-HSD1 at 0.6 μM. Coumarin derivative **6a** showed remarkable 76% and 100% 17β-HSD1 inhibition at 0.6 μM and 6 μM, respectively, and was the most potent 17β-HSD1 inhibitor arising from this study. The phenolic OH group of **6a** mimics the 3-OH group of E1, and this suggests that **6a** might work as a steroidal substrate mimetic, interacting in the same way with the polar amino acid residues in the substrate-binding region as for E1. The replacement of the 3-acetyl group with 3-ethoxycarbonyl group (compounds **11**−**14**) or leaving position 3 of the coumarin unsubstituted (compounds **19**−**21**) led to significant reductions in 17β-HSD1 inhibitory activity, suggesting that the 3-acetyl group is favorable for optimal interactions with the enzyme. None of the most potent inhibitors **4**, **5**, **6**, and **6a** showed inhibition of 17β-HSD2 at 60 μM, thereby confirming the excellent selectivity for 17β-HSD1 over 17β-HSD2 of inhibitors from this structural class.

Introduction of various *p*-substituted phenyl groups to position 6 of the coumarin scaffold did not result in potent inhibitors of 17β-HSD1, and the concomitant variation of substituents in position 3 did not result in significant differences in the inhibitory activities of this series (Table 3). These

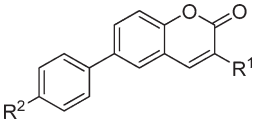
Table 2. Effects of 7-Phenyl Coumarin Derivatives on Inhibition of 17β-HSD1 and 17β-HSD2



			inhibition of 17β-HSD1 (%)[a]		inhibition of 17β-HSD2 (%)[a]		
compound	R^1	R^2	0.6 μM[b]	6 μM	0.6 μM	6 μM	60 μM
4	-$COCH_3$	-H	57	63	n.d.[c]	n.i.[d]	n.i.
5	-$COCH_3$	-Me	40	72	n.d.	n.i.	n.i.
6	-$COCH_3$	-OMe	48	86	n.i.	n.i.	n.i.
6a	-$COCH_3$	-OH	76	100	n.i.	n.i.	n.i.
7	-$COCH_3$	-Cl	12	17	n.d.	n.d.	n.d.
8	-$COCH_3$	-F	6	13	n.d.	n.d.	n.d.
11	-COOEt	-H	n.i.	7	n.d.	n.d.	n.d.
12	-COOEt	-Me	11	9	n.d.	n.d.	n.d.
13	-COOEt	-OMe	n.i.	1	n.d.	n.d.	n.d.
14	-COOEt	-Cl	n.i.	n.i.	n.d.	n.d.	n.d.
18	-H	-H	n.i.	10	n.d.	n.d.	n.d.
19	-H	-Me	10	29	n.d.	n.d.	n.d.
20	-H	-OMe	n.i.	10	n.d.	n.d.	n.d.
20a	-H	-OH	n.i.	43	n.d.	n.d.	n.d.
21	-H	-Cl	n.i.	9	n.d.	n.d.	n.d.

[a] Recombinant human enzyme overexpressed in *E. coli*, [^{3}H]E1 + E1 [67 nM] and NADPH [100 μM] for 17β-HSD1, [^{3}H]E2 + E2 [300 nM] and NAD^+ [100 μM] for 17β-HSD2; data are mean values of two determinations, with RSD less than 10%. [b] Concentration of inhibitor. [c] Not determined. [d] No inhibition.

Table 3. Effects of 6-Phenyl Coumarin Derivatives on Inhibition of 17β-HSD1



			inhibition of 17β-HSD1 (%)[a]	
compound	R^1	R^2	0.6 μM[b]	6 μM
23	-$COCH_3$	-H	n.i.[c]	n.i.
24	-$COCH_3$	-Me	n.i.	n.i.
25	-$COCH_3$	-OMe	n.i.	n.i.
26	-$COCH_3$	-Cl	n.i.	n.i.
27	-$COCH_3$	-F	15	59
29	-COOEt	-H	n.i.	21
30	-COOEt	-Me	6	16
31	-COOEt	-OMe	n.i	7
32	-COOEt	-Cl	10	14
33	-COOEt	-F	21	21
35	-H	-H	n.i.	18
36	-H	-Me	n.i.	n.i.
37	-H	-OMe	7	26
37a	-H	-OH	4	30
38	-H	-Cl	n.i.	10

[a] Recombinant human enzyme overexpressed in *E. coli*, [^{3}H]E1 + E1 [67 nM] and NADPH [100 μM]; data are mean values of two determinations, with RSD less than 15%. [b] Concentration of inhibitor. [c] No inhibition.

results lead to the conclusion that 6-phenyl coumarins do not bind to the active site of 17β-HSD1 with the high affinities of the 7-phenyl coumarins. The only exception here was compound **27**, which showed moderate inhibition of 17β-HSD1 at 6 μM and almost no inhibition at 0.6 μM; the rest of the compounds from this series were practically inactive.

The experimentally determined IC_{50} values and estimated K_i values for 17β-HSD1 inhibition of the most potent compounds **3**−**6** and **6a** are presented in Table 4. The K_i values were calculated by the Cheng-Prusoff equation,[60] presuming that these compounds are competitive reversible inhibitors. These results again show the importance of proton donor OH-group in **6a** for 17β-HSD1 inhibitory activity of the 3-acetyl-7-phenyl coumarin series. Compounds **3** and **6a** are potent inhibitors of 17β-HSD1 in the nanomolar range, with IC_{50} values of 360 and 270 nM, respectively. For the most potent inhibitor **6a**, the estimated K_i value is just about 2-fold higher than the K_m value for E1 (60 nM) that we experimentally determined with the same assay, which led us to conclusion that binding affinities of substrate and inhibitor **6a** toward 17β-HSD1 are comparable.

Table 4. IC_{50} Values for Compounds **3**−**6** and **6a** Determined on Bacterial Homogenates of Recombinant Human 17β-HSD1, and Calculated K_i Values

	17β-HSD1[a] (bacterial homogenate)	
compound	IC_{50}[b] (nM)	K_i[c] (nM)
3	360	173
4	1928	911
5	1237	585
6	1135	585
6a	270	143

[a] Recombinant human enzyme overexpressed in *E. coli*. [b] Data are mean values of three determinations, with RSD less than 10%. [c] K_i estimated from IC_{50} by Cheng-Prusoff equation for competitive inhibitor.[60]

In addition to selectivity against 17β-HSD2, 17β-HSD1 inhibitors to be used as potential drug therapies should not show high affinities toward ERα and ERβ, as the binding to these receptors can counteract their therapeutic efficiency. The relative binding affinities (RBAs) of the most potent 17β-HSD1 inhibitors **3**, **6**, and **6a** were determined using recombinant human ERs of both subtypes in a competitive binding assay, using recombinant human receptors and [^{3}H]-E2.[61] None of the coumarins tested showed any detectable affinity

Table 5. Binding affinities of selected compounds for recombinant human ERα and ERβ

	RBA[a] (%)	
compound	ER_α[b]	ER_β[b]
3	$<10^{-8}$	$<10^{-8}$
6	$<10^{-8}$	$<10^{-8}$
6a	$<10^{-8}$	$<10^{-8}$
Equilin	0.15	1.40

[a] RBA (relative binding affinity): E2, 100%, mean values of three determinations, with RSD less than 10%. [b] Recombinant human protein [0.9 nM], incubation with 9 nM [^{3}H]-E2 and inhibitor for 16 h.

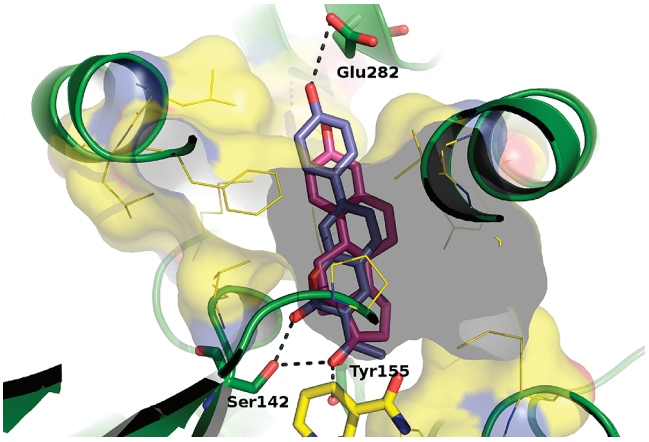


Figure 1. Docking of **6a** (blue) to the active site of the ternary 17β-HSD1 complex (PDB entry 1A27). Comparison of the binding modes between **6a** and E2 (magenta) is shown. Hydrogen bonds between **6a** and the polar residues are shown as dashed lines. The nicotinamide moiety of $NADP^+$ (yellow thick sticks) is also shown. Hydrophobic residues are shown as yellow spheres.

for the ERs (Table 5). Their binding affinities to the ERs were compared to the ER affinity of equilin, which is the equine E1 that is used in hormone replacement therapy.[59] As a side effect, this estrogen stimulates proliferation of human breast cancer cells via the ERs, although this stimulation is lower than that with E2.[23,62] It has also been reported that equilin shows a significantly higher affinity toward ERβ than ERα,[62] which was also confirmed in our assays. The RBAs toward the ERs of the coumarins and equilin tested are not comparable, and we can conclude that our compounds have no affinity for the ERs. These results suggest that our most promising coumarins do not have ER agonistic or antagonistic activities, which could counteract their potential therapeutic benefits.

Molecular Docking

The plausible binding modes of the 6-phenyl and 7-phenyl coumarin derivatives in the active site of 17β-HSD1 were assessed by molecular docking of selected compounds, using *FlexX 3.1*.[63] In the crystal structure of 17β-HSD1 (PDB entry 1A27),[64] the active site was defined as the area within 6.5 Å of the co-crystallized E2. In Figure 1, the predicted binding conformation of the 7-phenyl coumarin **6a** in the active site of 17β-HSD1 is shown. The inhibitor **6a** occupies a similar region to the co-crystallized E2, where the carbonyl oxygen of its 3-acetyl group overlaps closely with the C-17 oxygen of E2, thus showing a similar role for the forming of a bifurcated H-bond to the catalytic residues Ser142 and Tyr155,[13,15] while the oxygen of its lactone carbonyl group forms an additional H-bond to Ser142. On the opposite side of the substrate-binding

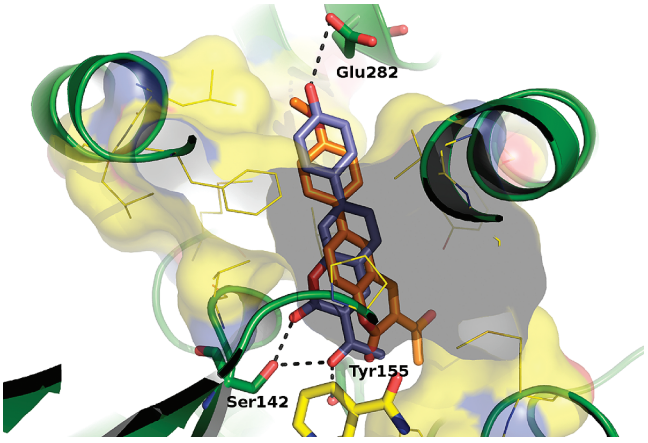


Figure 2. Docking of **6a** (blue) and **25** (orange) to the active site of the ternary 17β-HSD1 complex (PDB entry 1A27). For clarity, the structure of E2 has been removed from the complex. Hydrogen bonds between **6a** and the polar residues are shown as dashed lines. The nicotinamide moiety of $NADP^+$ (yellow thick sticks) is also shown. Hydrophobic residues are shown as yellow spheres.

region, the phenolic OH group of **6a** forms a H-bond with Glu282. The docking program did not predict H-bonding of the **6a** phenolic OH to His221, which has also been postulated to be important for substrate binding. The reason for this could be the 13 Å distance between the acetyl O and phenolic O in **6a**, which exceeds the 12 Å distance between O(C3) and O(C17) in E2, and thus prevents the correct orientation of the phenolic OH in **6a** for H-bonding to His221. Although some 17β-HSD1 crystal structures of ternary complexes[15,65] and kinetic experiments with 17β-HSD1 mutants[66,67] have not confirmed the role of Glu282 through the formation of a H-bond to the substrate 3-OH group, the published crystal structures of ternary complexes of 17β-HSD1 with the steroidal 17β-HSD1 inhibitors equilin[59] and E2B[31] clearly show that the phenolic 3-OH group of these steroidal inhibitors forms a H-bond with Glu282. A similar pose was also predicted for our inhibitor **6a**. The coumarin core of **6a**, which is accommodated in the hydrophobic pocket, overlaps closely with the B and C rings of E2, which suggests the formation of similar van der Waals contacts to the active-site lipophilic residues.[13] It appears that the intracyclic oxygen is not included in the formation of H-bonding with polar residues in the active site.

The absence of inhibitory activities for the 6-phenyl coumarin derivatives can also be explained by molecular docking (Figure 2). Due to their different geometry, 6-phenyl coumarins cannot be accommodated in the active site in a similar manner as the 7-phenyl coumarins, and consequently, H-bonds with the catalytic Ser142 and Tyr155 are not feasible. Compound **25** shows a distorted orientation when compared to **6a**, with both carbonyl groups pointing away from the catalytic residues, which prevents the formation of the H-bonds.

To further explain the SAR and devise rules for 17β-HSD1 inhibition by 3-acetyl-7-phenyl coumarins, a pharmacophore model was built (Figure 3). First, a superposition of active compounds was performed and pharmacopohoric points were detected and visualized. For potent inhibitory activity of this series of coumarins, the H-bond donor (A) that forms interaction with Glu282 and two H-bond acceptors interacting with Ser142 (B) and Tyr155 (C) are important. The H-bond acceptors B and C should be approximately 3 Å apart and 11 Å and 12 Å away from H-bond donor A, respectively. H-bond donor (A) and H-bond acceptors (B and C) should be

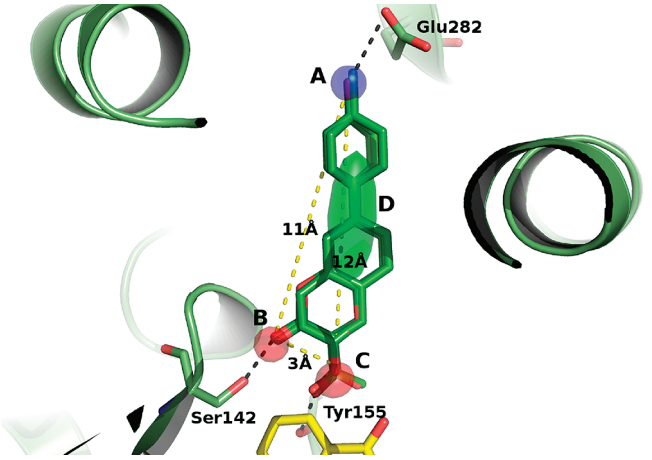


Figure 3. Pharmacophore model derived from SAR of coumarin derivatives in the active site of the ternary 17β-HSD1 complex (PDB entry 1A27). Proton donor group (A) is shown as a blue sphere, while the proton acceptor groups (B and C) are shown as red spheres. The lipophilic core (D) is shown as a green ellipsoid. Distances between proton donor and proton acceptor groups are shown as yellow dashed lines.

connected by the hydrophobic region (D) of appropriate size to interact with the central hydrophobic part of the active site. The proper distances between pharmacophoric points are presented in Figure 3.

Conclusions

The SM cross-coupling methodology was used for the generation of a library of 6-phenyl and 7-phenyl coumarin derivatives, as potential inhibitors of 17β-HSD1. We established clear structure−activity relationships, where 7-(4-substituted)-phenyl coumarin derivatives with an acetyl group in position 3 of the coumarin scaffold provided highly active inhibitors of 17β-HSD1, with the most potent compound **6a** showing an IC_{50} of 270 nM. Moving the phenyl substitution from position 7 to position 6 or altering the acetyl substituent on position 3 of the coumarins resulted in a drastic reduction in the 17β-HSD1 inhibitory activity. Docking studies have revealed that the 7-phenyl coumarin derivatives can occupy a similar binding region as E2, where the phenolic 4-OH group of **6a** can form polar interactions with Glu282, while on the other side, both the keto groups of the coumarin ring and the 3-acetyl substituent can form H-bonds with the residues of the catalytic triad. From SAR results, a simple pharmacophore model was established. None of the active 7-phenyl coumarins showed inhibition of 17β-HSD2 at 60 μM, which makes them exceptionally selective inhibitors for 17β-HSD1 over 17β-HSD2. Furthermore, none of the 7-phenyl coumarins tested showed detectable affinity toward either subtype of the ERs, which proved our rational design concepts for devoiding ER affinity. The absence of any ER affinity suggests that these inhibitors lack estrogenic and anti-estrogenic effects. The 7-phenyl coumarins described here demonstrate that the coumarin derivatives are an important structural class that can be used for the further development of potent and very selective nonsteroidal 17β-HSD1 inhibitors.

Experimental Section

Chemical Methods. The chemical names follow the IUPAC nomenclature. The starting chemicals were purchased from Acros, Aldrich, or Fluka, and were used without further purification. Dichloromethane was kept over calcium hydride and distilled immediately prior to use. Analytical TLC was performed on Merck 60 F_{254} silica gel plates (0.25 mm), using visualization with ultraviolet light, 2,4-dinitrophenyl hydrazine, and $FeCl_3$. Flash chromatography was carried out on silica gel 60 (particle size, 240−400 mesh). The melting points were determined on a Reichert hot stage microscope and are uncorrected. The IR spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrometer. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, on a Bruker AVANCE DPX_{300} spectrometer in $CDCl_3$ or DMSO-d_6 solutions, with TMS as the internal standard. The microanalyses were performed on a 240 C Perkin-Elmer C, H, N analyzer. The analyses indicated by the symbols of the elements were within ±0.4% of the theoretical values. The mass spectra were obtained using a VG-Analytical Autospec Q mass spectrometer. Reversed-phase high-performance liquid chromatography (HPLC) analyses were run on Agilent 1100 system (Agilent Technologies, Santa Clara, CA, USA) equipped with quaternary pump and multiple-wavelength detector using Agilent Eclipse Plus C18, 5 μm (150 × 4.6 mm) column. Compounds were dissolved in 50% acetonitrile/water at 0.1 mg/mL final concentration, and 10 μL was injected onto the column. Acetonitrile was used as an organic modifier and 0.1% TFA in water as an aqueous buffer. The elution was performed using linear gradient from 20% to 80% of acetonitrile over 15 min with 1.0 mL/min flow rate and monitored at 210 nm. The relative purity of all tested compounds was above 95.0%.

Method A. A general procedure for preparation of compounds **1** and **34**:[53] The corresponding salicylaldehyde (1 equiv) and ethyl acetoacetate (1 equiv) were dissolved in ethanol. Piperidine (0.02 equiv) was added, and the mixture was refluxed for 4 h. Filtration of the cooled mixture and the subsequent washing of product with cold ethanol provided analytically pure crystals.

Method B. A general procedure for preparation of compounds **2**, **6a**, **16**, and **20a**:[54] The methoxyaryl compound and $AlCl_3$ (3.3 equiv) were suspended in toluene and stirred under reflux for 3 h. Toluene was removed in vacuo, and the residue was purified by flash chromatography (0−5% MeOH in dichloromethane).

Method C. A general procedure for preparation of compounds **3**, **10**, and **17**:[55] a suspension of the corresponding phenol in dry dichloromethane and anhydrous pyridine (1.4 equiv) was cooled to −10 °C. Trifluormethanesulfonic anhydride (1.2 equiv) was added dropwise over a period of 15 min, under Ar. After 1 h, the reaction was quenched with a saturated solution of aqueous $NaHCO_3$ in an ice bath (caution: exothermic) and washed three times with water. The organic layer was filtered over silica beads, the solvent was evaporated in vacuo, and the dry residue was recrystallized from ethyl acetate.

Method D. SM cross-coupling general procedure for preparation of compounds **4**−**8**, **11**−**14**, and **18**−**21**:[52] a mixture of the 7-triflylcoumarin derivative, the appropriate phenylboronic acid (1.6 equiv), potassium carbonate (2.5 equiv), and tetrakis-(triphenylphosphine) palladium (4.5% by weight of triflate) in deoxygenated toluene was stirred at 100 °C overnight, under Ar. After cooling to room temperature, the mixture was concentrated in vacuo to dryness, and the residue was purified by flash chromatography (0−40% ethyl acetate in hexane).

Method E. SM cross-coupling general procedure for preparation of compounds **23**−**27**, **29**−**33**, and **35**−**38**:[52] a mixture of the 6-bromocoumarin derivative, the appropriate phenylboronic acid (1.6 equiv), potassium carbonate (2.5 equiv), and tetrakis(triphenylphosphine) palladium (4.5% by weight of bromide) in deoxygenated toluene was stirred at 100 °C overnight, under Ar. After cooling to room temperature, the mixture was concentrated in vacuo to dryness, and the residue was purified by flash chromatography (0−20% ethyl acetate in hexane).

Method F. A general procedure for the preparation of compounds **9** and **28**:[56] the mixture of the appropriate derivative of 2-hydroxybenzaldehyde and diethyl malonate (1.1 equiv) in ethanol, piperidine (0.1 equiv), and glacial acetic acid (0.0005 equiv) was heated under reflux for 3 h. After the addition of hot water (60 °C) and cooling of the solution, the crystals of the product were collected by filtration.

3-Acetyl-7-methoxy-2*H*-chromen-2-one (1). The title compound was prepared from 2-hydroxy-4-methoxy benzaldehyde (3.0 g, 19.7 mmol) in ethanol (100 mL), according to method A. Yield: 4.30 g (93%), yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.05 (s, 1H), 7.55 (d, J = 8.70 Hz, 1H), 6.91 (dd, J = 8.70 Hz, 2.3 Hz, 1H), 6.84 (d, J = 2.30 Hz, 1H), 3.93 (s, 3H), 2.71 (s, 3H) ppm. MS (ESI): m/z 219.0 $[M+H]^+$.

3-Acetyl-7-hydroxy-2*H*-chromen-2-one (2). The title compound was prepared from **1** (5.0 g, 22.9 mmol) in toluene (250 mL), according to method B. The product was purified by flash chromatography (0−5% MeOH in dichloromethane). Yield: 3.21 g (68%), yellow powder. ^{1}H NMR (300 MHz, DMSO) δ: 11.10 (bs, 1H), 8.58 (s, 1H), 7.78 (d, J = 8.4 Hz, 1H), 6.85 (dd, J = 8.6 Hz, 2.4 Hz, 1H), 6.75 (d, J = 2.4 Hz, 1H), 2.54 (s, 3H) ppm. MS (ESI): m/z 205.0 $[M+H]^+$.

3-Acetyl-2-oxo-2*H*-chromen-7-yl trifluoromethanesulfonate (3). The title compound was prepared from **2** (8.20 g, 40.2 mmol) in dry dichloromethane (200 mL), according to method C. Yield: 10.01 g (86%), yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.50 (s, 1H), 7.78 (d, J = 8.7 Hz, 1H), 7.35 (d, J = 2.1 Hz, 1H), 7.30 (partly overlapped by the solvent signal, 1H), 2.74 (s, 3H) ppm. HRMS (ESI) calculated for $C_{12}H_8F_3O_6S$ $[MH]^+$ 336.9994, found 336.9994.

3-Acetyl-7-phenyl-2*H*-chromen-2-one (4). The title compound was prepared from **3** (500 mg, 1.49 mmol), phenylboronic acid (290 mg, 2.38 mmol), potassium carbonate (514 mg, 3.72 mmol), and tetrakis(triphenylphosphine) palladium (22.5 mg, 25 μmol) in 20 mL toluene, according to method D. The product was purified by flash column chromatography (ethyl acetate in hexane: 0−15%). Yield: 134 mg (34%), yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.57 (s, 1H), 7.76−7.745 (m, 8H), 2.77 (s, 3H) ppm. HRMS (ESI) calculated for $C_{17}H_{12}O_3Na$ $[MNa^+]$ 287.0684, found 287.0686.

3-Acetyl-7-*p*-tolyl-2*H*-chromen-2-one (5). The title compound was prepared from **3** (1.0 g, 2.97 mmol), *p*-tolylboronic acid (647 mg, 4.76 mmol), potassium carbonate (1.03 g, 7.44 mmol), and tetrakis(triphenylphosphine) palladium (45 mg, 50 μmol) in 50 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 140 mg (17%), yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.56 (s, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.61−7.56 (m, 4H), 7.33 (d, 2H, J = 7.9 Hz, 2H), 2.76 (s, 3H), 2.45 (s, 3H) ppm. HRMS (ESI) calculated for $C_{18}H_{15}O_3$ $[MH]^+$ 279.1021, found 279.1009.

3-Acetyl-7-(4-methoxyphenyl)-2*H*-chromen-2-one (6). The title compound was prepared from **3** (500 mg, 1.49 mmol), *p*-methoxyphenylboronic acid (362 mg, 2.38 mmol), potassium carbonate (514 mg, 3.72 mmol), and tetrakis(triphenylphosphine) palladium (22.5 mg, 25 μmol) in 20 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate/hexane: 0−15%). Yield: 360 mg (72%), yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.55 (s, 1H), 7.70−7.56 (m, 5H), 7.05 (d, J = 8.7 Hz, 2H), 3.90 (s, 3H), 2.76 (s, 3H) ppm. HRMS (ESI) calculated for $C_{18}H_{15}O_4$ $[MH]^+$ 295.0970, found 295.0980.

3-Acetyl-7-(4-hydroxyphenyl)-2*H*-chromen-2-one (6a). The title compound was prepared from **6** (200 mg, 0.68 mmol) in toluene (10 mL), according to method B. The product was purified by flash chromatography (0−3% MeOH in dichloromethane). Yield: 110 mg (59%), yellow crystals. ^{1}H NMR (300 MHz, DMSO) δ: 9.86 (s, 1H), 8.68 (s, 1H), 7.96 (d, J = 8.7 Hz, 1H), 7.73−7.70 (m, 4H), 6.90 (d, J = 8.6 Hz, 2H), 2.60 (s, 3H) ppm. ^{13}C NMR (DMSO) δ: 195.8, 159.6, 159.6, 156.3, 147.8, 147.1, 132.0, 129.5, 129.3, 123.9, 123.4, 117.3, 116.9, 113.2, 30.9 ppm. HRMS (ESI) calculated for $C_{17}H_{13}O_4$ $[MH]^+$ 281.0814, found 281.0809; anal. ($C_{17}H_{12}O_4$) C, H, N.

3-Acetyl-7-(4-chlorophenyl)-2*H*-chromen-2-one (7). The title compound was prepared from **3** (250 mg, 0.74 mmol), *p*-chlorophenylboronic acid (186 mg, 1.19 mmol), potassium carbonate (257 mg, 1.86 mmol), and tetrakis(triphenylphosphine) palladium (11 mg, 12 μmol) in 10 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 74 mg (33%), yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.55 (s, 1H, H-4), 7.73 (d, J = 8.1 Hz, 1H), 7.61−7.48 (m, 6H), 2.76 (s, 3H) ppm. HRMS (ESI) calculated for $C_{17}H_{12}ClO_3$ $[MH]^+$ 299.0475, found 299.0470; anal. ($C_{17}H_{12}ClO_3$) C, H, N.

3-Acetyl-7-(4-fluorophenyl)-2*H*-chromen-2-one (8). The title compound was prepared from **3** (250 mg, 0.74 mmol), *p*-fluorophenylboronic acid (176 mg, 1.19 mmol), potassium carbonate (257 mg, 1.86 mmol), and tetrakis(triphenylphosphine) palladium (11 mg, 12 μmol) in 10 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 20 mg (10%) gray powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.70 (s, 1H), 7.85−7.79 (m, 2H), 7.58−7.54 (m, 2H), 7.46 (d, J = 8.4 Hz, 1H), 7.23−7.17 (m, 2H), 2.77 (s, 3H) ppm. HRMS (ESI) calculated for $C_{17}H_{12}FO_3$ $[MH]^+$ 283.0770, found 299.0773.

Ethyl 7-Hydroxy-2-oxo-2*H*-chromene-3-carboxylate (9). The title compound was prepared from 2,4-dihydroxybenzaldehyde (4.0 g, 29.0 mmol) in ethanol (20 mL), according to method F. Yield: 3.14 g (46%), white crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 11.05 (s, 1H), 8.67 (s, 1H), 7.75 (d, J = 8.6 Hz, 1H), 6.84 (dd, J = 8.6 Hz, 2.3 Hz, 1H), 6.73 (d, J = 2.1 Hz, 1H), 4.26 (q, J = 7.1 Hz, 2H), 1.30 (t, J = 7.1 Hz, 3H) ppm. MS (ESI): m/z 235.1 $[M+H]^+$.

Ethyl 2-Oxo-7-(trifluoromethylsulfonyloxy)-2*H*-chromene-3-carboxylate (10). The title compound was prepared from **9** (2.0 g, 8.54 mmol) in dry dichloromethane (40 mL), according to method C. Yield: 1.61 g (52%), white crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.52 (s, 1H), 7.74 (d, J = 8.5 Hz, 1H), 7.30−7.33 (m, 2H), 4.45 (q, J = 7.1 Hz, 2H); 1.44 (t, J = 7.1 Hz, 3H) ppm. HRMS (ESI) calculated for $C_{13}H_{10}F_3O_7S$ $[MH]^+$ 367.0099, found 367.0105.

Ethyl 2-Oxo-7-phenyl-2*H*-chromene-3-carboxylate (11). The title compound was prepared from **10** (200 mg, 0.55 mmol), phenylboronic acid (107 mg, 0.87 mmol), potassium carbonate (189 mg, 1.37 mmol), and tetrakis(triphenylphosphine) palladium (9 mg, 10 μmol) in 10 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate in hexane: 0−25%). Yield: 148 mg (92%), pale yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.58 (s, 1H), 7.65−7.70 (m, 3H), 7.58−7.61 (m, 2H), 7.45−7.55 (m, 3H), 4.45 (q, J = 7.1 Hz, 2H), 1.45 (t, J = 7.1 Hz, 3H) ppm. HRMS (ESI) calculated for $C_{18}H_{15}O_4$ $[MH]^+$ 295.0970, found 295.0974.

Ethyl 2-Oxo-7-*p*-tolyl-2*H*-chromene-3-carboxylate (12). The title compound was prepared from **10** (200 mg, 0.55 mmol), *p*-tolylboronic acid (119 mg, 0.88 mmol), potassium carbonate (187 mg, 1.37 mmol), and tetrakis(triphenylphosphine) palladium (9 mg, 10 μmol) in 10 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate in hexane: 0−20%). Yield: 116 mg (69%), pale yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.57 (s, 1H), 7.66 (d, J = 8.6 Hz, 1H), 7.55−7.60 (m, 4H), 7.33 (d, J = 7.9 Hz, 2H), 4.45 (q, J = 7.1 Hz, 2H), 2.36 (s, 3H), 1.44 (t, J = 7.1 Hz, 3H) ppm. HRMS (ESI) calculated for $C_{19}H_{17}O_4$ $[MH]^+$ 309.1127, found 309.1128.

Ethyl 7-(4-Methoxyphenyl)-2-oxo-2*H*-chromene-3-carboxylate (13). The title compound was prepared from **10** (600 mg, 1.64 mmol), *p*-methoxyphenylboronic acid (399 mg, 2.62 mmol), potassium carbonate (566 mg, 4.10 mmol), and tetrakis(triphenylphosphine) palladium (27 mg, 30 μmol) in 30 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate in hexane: 0−25%).

Yield: 204 mg (46%), yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.56 (s, 1H), 7.54−7.66 (m, 5H), 7.04 (d, J = 8.8 Hz, 2H), 4.45 (q, J = 7.1 Hz, 2H), 3.82 (s, 3H), 1.44 (t, J = 7.1 Hz, 3H) ppm. HRMS (ESI) calculated for $C_{19}H_{17}O_5$ $[MH]^+$ 325.1076, found 325.1077.

Ethyl 7-(4-Chlorophenyl)-2-oxo-2*H*-chromene-3-carboxylate (14). The title compound was prepared from **10** (200 mg, 0.55 mmol), *p*-chlorophenylboronic acid (136.7 mg, 0.87 mmol), potassium carbonate (189 mg, 1.37 mmol), and tetrakis(triphenylphosphine) palladium (9 mg, 10 μmol) in 10 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate in hexane: 0−30%). Yield: 142.7 mg (80%), pale yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.57 (s, 1H), 7.69 (d, J = 8.5 Hz, 1H), 7.48−7.60 (m, 6H), 4.45 (q, J = 7.1 Hz, 2H), 1.44 (t, J = 7.1 Hz, 3H) ppm. HRMS (ESI) calculated for $C_{18}H_{14}O_4Cl$ $[MH]^+$ 329.0581, found 329.0582.

7-Methoxy-2*H*-chromen-2-one (15). The title compound was prepared by a modified procedure for preparation of coumarins using the Wittig reaction.[57] A mixture of 2-hydroxy-4-methoxybenzaldehyde (1.0 g, 6.58 mmol) and [(methoxycarbonyl)-methylene]triphenylphosphorane (2.75 g, 7.89 mmol) was heated in *N*,*N*-diethylaniline (65 mL) at 210 °C for 2 h. After cooling to room temperature, the reaction mixture was diluted with 5% HCl solution (70 mL) and extracted with ether (3 × 50 mL). The combined organic phases were dried over Na_2SO_4, filtered, and evaporated in vacuo. The oily residue was purified by flash column chromatography (0−20% ethylacetate in hexane). Yield: 697 mg (60%), brown powder. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.65 (d, J = 9.5 Hz, 1H), 7.39 (d, J = 8.3 Hz, 1H), 6.84−6.88 (m, 2H), 6.27 (d, J = 9.5 Hz, 1H), 3.90 (s, 3H) ppm. MS (ESI): m/z 177.1 $[M+H]^+$.

7-Hydroxy-2*H*-chromen-2-one (16). The title compound was prepared from **15** (492 mg, 2.79 mmol) in toluene (25 mL), according to method B. The product was purified by flash chromatography (0−1% MeOH in dichloromethane). Yield: 390 mg (86%), brown powder. ^{1}H NMR (300 MHz, DMSO) δ: 7.93 (d, J = 9.4 Hz, 1H); 7.53 (d, J = 8.5 Hz, 1H), 6.79 (dd, J = 8.5 Hz, 2.3 Hz, 1H), 6.71 (d, J = 2.3 Hz, 1H), 6.20 (d, J = 9.4 Hz, 1H) ppm. MS (ESI) m/z 161.0 $[M+H]^+$.

2-Oxo-2*H*-chromen-7-yl trifluoromethanesulfonate (17). The title compound was prepared from **16** (5.0 g, 30.7 mmol) in dry dichloromethane (100 mL), according to method C. Yield: 8.0 g (88%), white crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.74 (d, J = 9.6 Hz, 1H); 7.60 (d, J = 8.6 Hz, 1H), 7.30 (d, J = 2.3 Hz, 1H), 7.24 (dd, J = 8.6, 2.4 Hz, 1H), 6.51 (d, J = 9.6 Hz, 1H) ppm. HRMS (ESI) calculated for $C_{10}H_6O_5F_3S$ $[MH]^+$ 294.9888, found 294.9886.

7-Phenyl-2*H*-chromen-2-one (18). The title compound was prepared from **17** (200 mg, 0.68 mmol), phenylboronic acid (133 mg, 1.09 mmol), potassium carbonate (235 mg, 1.70 mmol), and tetrakis(triphenylphosphine) palladium (9 mg, 10 μmol) in 10 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate in hexane: 0−40%). Yield: 143 mg (95%), pale brown crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.75 (d, J = 9.5 Hz, 1H), 7.64−7.67 (m, 2H), 7.42−7.58 (m, 6H), 6.45 (d, J = 9.5 Hz, 1H) ppm. HRMS (ESI) calculated for $C_{15}H_{11}O_2$ $[MH]^+$ 223.0759, found 223.0760.

7-(*p*-Tolyl)-2*H*-chromen-2-one (19). The title compound was prepared from **17** (250 mg, 0.85 mmol), *p*-tolylboronic acid (185 mg, 1.36 mmol), potassium carbonate (294 mg, 2.13 mmol), and tetrakis(triphenylphosphine) palladium (11 mg, 12 μmol) in 12 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate in hexane: 0−25%). Yield: 104 mg (52%), white crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.74 (d, J = 9.5 Hz, 1H), 7.54−7.56 (m, 5H), 7.31 (d, J = 8.0 Hz, 2H), 6.43 (d, J = 9.5 Hz, 1H), 2.44 (s, 3H) ppm. HRMS (ESI) calculated for $C_{16}H_{13}O_2$ $[MH]^+$ 237.0916, found 237.0905.

7-(4-Methoxyphenyl)-2*H*-chromen-2-one (20). The title compound was prepared from **17** (600 mg, 2.04 mmol), *p*-methoxyphenylboronic acid (496 mg, 3.26 mmol), potassium carbonate (705 mg, 5.10 mmol), and tetrakis(triphenylphosphine) palladium (27 mg, 29 μmol) in 30 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate in hexane: 0−40%). Yield: 492 mg (96%), gray crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.74 (d, J = 9.0 Hz, 1H), 7.59−7.61 (m, 2H), 7.51−7.53 (m, 3H), 7.02−7.05 (m, 2H), 6.42 (d, J = 9.6 Hz, 1H), 3.89 (s, 3H) ppm. HRMS (ESI) calculated for $C_{16}H_{13}O_2$ $[MH]^+$ 253.0865, found 253.0862.

7-(4-Hydroxyphenyl)-2*H*-chromen-2-one (20a). The title compound was prepared from **20** (458 mg, 1.82 mmol) in toluene (23 mL), according to method B. The product was purified by flash chromatography (0−1% MeOH in dichloromethane). Yield: 283 mg (66%), pale brown crystals. ^{1}H NMR (300 MHz, DMSO) δ: 9.75 (s, 1H), 8.07 (d, J = 9.5 Hz, 1H), 7.73 (d, J = 8.6 Hz, 1H), 7.61−7.66 (m, 4H), 6.88 (d, J = 8.6 Hz, 2H), 6.44 (d, J = 9.5 Hz, 1H) ppm. HRMS (ESI) calculated for $C_{15}H_{11}O_3$ $[MH]^+$ 239.0708, found 239.0711.

7-(4-Chlorophenyl)-2*H*-chromen-2-one (21). The title compound was prepared from **17** (200 mg, 0.68 mmol), *p*-chlorophenylboronic acid (170 mg, 1.09 mmol), potassium carbonate (235 mg, 1.70 mmol), and tetrakis(triphenylphosphine) palladium (9 mg, 10 μmol) in 10 mL toluene, according to method D. The product was purified by flash chromatography (ethyl acetate in hexane: 0−25%). Yield: 85 mg (62%), gray crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.75 (d, J = 9.6 Hz, 1H), 7.46−7.59 (m, 7H), 6.46 (d, J = 9.6 Hz, 1H) ppm. HRMS (ESI) calculated for $C_{15}H_{10}O_2Cl$ $[MH]^+$ 257.0369, found 257.0362.

3-Acetyl-6-bromo-2*H*-chromen-2-one (22). The title compound was prepared from 5-bromosalicylaldehyde (6.0 g, 29.9 mmol) in 200 mL ethanol, according to method A. Yield: 7.50 g (81%), pale yellow crystals. ^{1}H NMR (300 MHz, DMSO) δ: 8.60 (s, 1H), 8.22 (d, J = 2.4 Hz, 1H), 7.89 (dd, J = 9.0 Hz, 2.4 Hz, 1H), 7.45 (d, J = 9 Hz, 1H), 2.58 (s, 3H) ppm. MS (ESI): m/z 267.0 $[M+H]^+$.

3-Acetyl-6-phenyl-2*H*-chromen-2-one (23). The title compound was prepared from **22** (400 mg, 1.50 mmol), phenylboronic acid (292 mg, 2.40 mmol), potassium carbonate (518 mg, 3.74 mmol), and tetrakis(triphenylphosphine) palladium (18 mg, 20 μmol) in 10 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 160 mg (40%), yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.59 (s, 1H), 7.89 (dd, J = 8.6 Hz, 2.1 Hz, 1H), 7.84 (d, J = 2.1 Hz, 1H), 7.62−7.59 (m, 2H), 7.54 (m, 4H), 2.77 (s, 3H) ppm. HRMS (ESI) calculated for $C_{17}H_{13}O_3$ 265.0865 $[MH]^+$, found 265.0871.

3-Acetyl-6-*p*-tolyl-2*H*-chromen-2-one (24). The title compound was prepared from **22** (400 mg, 1.50 mmol), *p*-tolylboronic acid (326 mg, 2.40 mmol), potassium carbonate (518 mg, 3.74 mmol), and tetrakis(triphenylphosphine) palladium (18 mg, 20 μmol) in 10 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 143 mg (34%), yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.58 (s, 1H), 7.88 (dd, J = 8.7 Hz, 2.1 Hz, 1H), 7.82 (d, J = 2.1 Hz, 1H), 7.50 (d, J = 8.1 Hz, 2H), 7.45 (d, J = 8.7 Hz, 1H), 7.31 (d, J = 7.8 Hz, 2H), 2.77 (s, 3H), 2.44 (s, 3H) ppm. HRMS (ESI) calculated for $C_{18}H_{15}O_3$ 279.1021 $[MH]^+$, found 279.1023.

3-Acetyl-6-(4-methoxyphenyl)-2*H*-chromen-2-one (25). The title compound was prepared from **22** (400 mg, 1.50 mmol), 4-methoxyphenylboronic acid (364 mg, 2.40 mmol), potassium carbonate (518 mg, 3.74 mmol), and tetrakis(triphenylphosphine) palladium (18 mg, 20 μmol) in 10 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 110 mg (25%), yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.57 (s, 1H), 7.85 (dd, J = 8.7 Hz, 2.1 Hz, 1H), 7.79 (d, J = 2.1 Hz, 1H), 7.53 (d, J = 8.7 Hz, 2H), 7.44 (d, J = 8.7 Hz, 1H), 7.03 (d, J = 8.7 Hz, 2H),

3.89 (s, 3H), 2.76 (s, 3H) ppm. HRMS (ESI) calculated for $C_{18}H_{15}O_4$ 295.0970 $[MH]^+$, found 295.0965.

3-Acetyl-6-(4-chlorophenyl)-2*H*-chromen-2-one (26). The title compound was prepared from **22** (250 mg, 0.94 mmol), 4-chlorophenylboronic acid (228 mg, 1.50 mmol), potassium carbonate (323 mg, 2.34 mmol), and tetrakis(triphenylphosphine) palladium (11 mg, 12 μmol) in 10 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 87 mg (31%), yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.57 (s, 1H), 7.85 (dd, J = 8.7 Hz, 2.1 Hz, 1H), 7.81 (d, J = 2.1 Hz, 1H), 7.55−7.46 (m, 5H), 2.77 (s, 3H) ppm. HRMS (ESI) calculated for $C_{17}H_{11}ClO_3Na$ 321.0294 $[MNa]^+$, found 321.0287.

3-Acetyl-6-(4-fluorophenyl)-2*H*-chromen-2-one (27). The title compound was prepared from **22** (250 mg, 0.94 mmol), 4-fluorphenylboronic acid (210 mg, 1.50 mmol), potassium carbonate (323 mg, 2.34 mmol), and tetrakis(triphenylphosphine) palladium (11 mg, 12 μmol) in 10 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 110 mg (44%), brown powder. ^{1}H NMR (300 MHz, DMSO) δ: 8.70 (s, 1H), 8.02 (d, J = 8.1 Hz, 1H), 7.93−7.88 (m, 2H), 7.79−7.74 (m, 2H), 7.39−7.33 (m, 2H), 2.60 (s, 3H) ppm. HRMS (ESI) calculated for $C_{17}H_{12}FO_3$ 283.0770 $[MH]^+$, found 283.0775.

Ethyl 6-Bromo-2-oxo-2*H*-chromene-3-carboxylate (28). The title compound was prepared from 5-bromo-2-hydroxybenzaldehyde (10.0 g, 49.75 mmol) in 200 mL ethanol, according to method F. Yield: 12.0 g (81%), white crystals. ^{1}H NMR (300 MHz, DMSO) δ: 8.69 (s, 1H), 8.16 (bs, 1H), 7.86 (dd, J = 8.8 Hz, 0.6 Hz, 1H), 7.40 (d, J = 8.8 Hz, 1H), 4.31 (q, J = 7.1 Hz, 2H), 1.31 (t, J = 7.1 Hz, 3H). HRMS (ESI) calculated for $C_{12}H_9BrO_4Na$ 318.9582 $[MNa]^+$, found 318.9587.

Ethyl 2-Oxo-6-phenyl-2*H*-chromene-3-carboxylate (29). The title compound was prepared from **28** (2.00 g, 6.73 mmol), phenylboronic acid (1.31 g, 10.77 mmol), potassium carbonate (2.33 g, 16.83 mmol), and tetrakis(triphenylphosphine) palladium (90 mg, 101 μmol) in 50 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%) and recrystallized from ethylacetate/hexane. Yield: 650 mg (33%), yellow crystals. ^{1}H NMR (300 MHz, DMSO) δ: 8.82 (s, 1H), 8.26 (d, J = 2.1 Hz, 1H), 8.05 (dd, J = 8.7 Hz, 2.1 Hz, 1H), 7.75−7.72 (m, 2H), 7.54−7.49 (m, 3H), 7.44−7.39 (m, 1H), 4.32 (q, J = 6.9 Hz, 2H), 1.33 (t, J = 6.9 Hz, 3H) ppm. HRMS (ESI) calculated for $C_{18}H_{15}O_4$ 295.0970 $[MH]^+$, found 295.0973; anal. ($C_{18}H_{14}O_4$) C, H, N.

Ethyl 2-Oxo-6-*p*-tolyl-2*H*-chromene-3-carboxylate (30). The title compound was prepared from **28** (2.00 g, 6.73 mmol), *p*-tolylboronic acid (1.46 g, 10.77 mol), potassium carbonate (2.33 g, 16.83 mmol), and tetrakis(triphenylphosphine) palladium (90 mg, 101 μmol) in 50 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 493 mg (24%), gray crystals. ^{1}H NMR (300 MHz, DMSO) δ: 8.81 (s, 1H), 8.23 (d, J = 1.8 Hz, 1H), 8.02 (dd, J = 8.7 Hz, 1.8 Hz, 1H), 7.62 (d, J = 8.1 Hz, 2H), 7.51 (d, J = 8.7 Hz, 1H), 7.32 (d, J = 8.1 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 2.36 (s, 3H), 1.33 (t, J = 7.1 Hz, 3H) ppm. HRMS (ESI) calculated for $C_{19}H_{16}O_4Na$ 331.0946 $[MNa]^+$, found 331.0948.

Ethyl 6-(4-Methoxyphenyl)-2-oxo-2*H*-chromene-3-carboxylate (31). The title compound was prepared from **28** (2.00 g, 6.73 mmol), 4-methoxyphenylboronic acid (1.64 g, 10.77 mmol), potassium carbonate (2.33 g, 16.83 mmol), and tetrakis(triphenylphosphine) palladium (90 mg, 101 μmol) in 50 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield 1.47 g (67%), yellow crystals. ^{1}H NMR (300 MHz, DMSO) δ: 8.80 (s, 1H), 8.20 (d, J = 2.3 Hz, 1H), 8.00 (dd, J = 8.7 Hz, 2.3 Hz, 1H), 7.67 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.7 Hz, 1H), 7.07 (d, J = 8.8 Hz, 2H), 4.32 (q, J = 7.1 Hz, 2H), 3.82 (s, 3H), 1.33 (t, J = 7.1 Hz, 3H) ppm. HRMS (ESI) calculated for $C_{19}H_{16}O_5Na$ 347.0895 $[MNa]^+$, found 347.0891; anal. ($C_{19}H_{16}O_5$) C, H, N.

Ethyl 6-(4-Chlorophenyl)-2-oxo-2*H*-chromene-3-carboxylate (32). The title compound was prepared from **28** (250 mg, 0.84 mmol), 4-chlorophenylboronic acid (205 mg, 1.35 mmol), potassium carbonate (291 mg, 2.10 mmol), and tetrakis(triphenylphosphine) palladium (11.3 mg, 13 μmol) in 10 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 170 mg (62%), white crystals. ^{1}H NMR (300 MHz, DMSO) δ: 8.80 (s, 1H), 8.28 (d, J = 2.3 Hz, 1H), 8.06 (dd, J = 8.7 Hz, 2.3 Hz, 1H), 7.76 (d, J = 8.6 Hz, 2H), 7.58 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 8.7 Hz, 1H), 4.32 (q, J = 7.1 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H) ppm. HRMS (ESI) calculated for $C_{18}H_{13}ClO_4Na$ 351.0400 $[MNa]^+$, found 351.0407; anal. ($C_{18}H_{13}ClO_4$) C, H, N.

Ethyl 6-(4-Fluorophenyl)-2-oxo-2*H*-chromene-3-carboxylate (33). The title compound was prepared from **28** (250 mg, 0.84 mmol), 4-fluorophenylboronic acid (188 mg, 1.35 mmol), potassium carbonate (291 mg, 2.10 mmol), and tetrakis(triphenylphosphine) palladium (11 mg, 13 μmol) in 10 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 142 mg (54%), white crystals. ^{1}H NMR (300 MHz, DMSO) δ: 8.80 (s, 1H), 8.24 (d, J = 2.3 Hz, 1H), 8.03 (dd, J = 8.7 Hz, 2.3 Hz, 1H), 7.77 (m, 2H), 7.52 (d, J = 8.7 Hz, 1H), 7.35 (m, 2H), 4.32 (q, J = 7.1 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H) ppm. HRMS (ESI) calculated for $C_{18}H_{14}FO_4$ 313.0876 $[MH]^+$, found 313.0873; anal. ($C_{18}H_{13}FO_4$) C, H, N.

6-Bromo-2*H*-chromen-2-one (34). The title compound was prepared by a modified procedure for Perkin condensation.[58] 5-Bromo-2-hydroxybenzaldehyde (3.00 g, 14.92 mmol), potassium carbonate (2.27 g, 16.42 mmol), and acetic anhydride (9.10 g, 89.14 mmol) were mixed in *N*,*N*-dimethylformamide. The slurry was heated to 70 °C, and water (0.3 mL) was added. The mixture was then heated to 120 °C and mixed overnight. After cooling the solution to 30 °C, water (30 mL) was added and the resulting precipitate was filtered, dried, and purified with flash chromatography (ethylacetate in hexane: 0−18%). Yield: 493 mg (15%), white crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.62−7.70 (m, 3H), 7.25 (d, J = 9.4 Hz, 1H), 6.49 (d, J = 9.6 Hz, 1H). HRMS (ESI) calculated for $C_9H_6O_2Br$ 224.9551 $[MH]^+$, found 224.9558.

6-Phenyl-2*H*-chromen-2-one (35). The title compound was prepared from **34** (100 mg, 0.44 mmol), phenylboronic acid (87 mg, 0.71 mmol), potassium carbonate (153 mg, 1.11 mmol), and tetrakis(triphenylphosphine) palladium (4.5 mg, 5 μmol) in 5 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−10%). Yield: 82 mg (83%), brown crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.76−7.80 (m, 2H), 7.69 (d, J = 2.2 Hz, 1H), 7.59−7.62 (m, 2H), 7.39−7.52 (m, 4H), 6.49 (d, J = 9.5 Hz, 1H) ppm. HRMS (ESI) calculated for $C_{15}H_{12}O_2$ 223.0759 $[MH]^+$, found 223.0763.

6-(*p*-Tolyl)-2*H*-chromen-2-one (36). The title compound was prepared from **34** (100 mg, 0.44 mmol), phenylboronic acid (97 mg, 0.71 mmol), potassium carbonate (153 mg, 1.11 mmol), and tetrakis(triphenylphosphine) palladium (4.5 mg, 5 μmol) in 5 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−15%). Yield: 69 mg (65%), white crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.74−7.79 (m, 2H), 7.67 (d, J = 2.0 Hz, 1H), 7.50 (d, J = 8.0 Hz, 2H), 7.41 (d, J = 8.6 Hz, 1H), 7.30 (d, J = 8.0 Hz, 2H), 6.48 (d, J = 9.5 Hz, 1H), 2.43 (s, 3H) ppm. HRMS (ESI) calculated for $C_{16}H_{13}O_2$ 237.0916 $[MH]^+$, found 237.0922.

6-(4-Methoxyphenyl)-2*H*-chromen-2-one (37). The title compound was prepared from **34** (340 mg, 1.51 mmol), *p*-methoxyphenylboronic acid (370 mg, 2.43 mmol), potassium carbonate (525 mg, 3.80 mmol), and tetrakis(triphenylphosphine) palladium (15 mg, 17 μmol) in 17 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−20%). Yield: 299 mg (78%), pale yellow crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.77 (d, J = 9.6 Hz,

1H), 7.73 (dd, J = 8.7 Hz, 2.0 Hz, 1H), 7.63 (d, J = 2.0 Hz, 1H), 7.53 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 8.6 Hz, 1H), 7.02 (d, J = 8.7 Hz, 2H), 6.47 (d, J = 9.5 Hz, 1H), 3.88 (s, 3H) ppm. HRMS (ESI) calculated for $C_{16}H_{13}O_3$ 253.0865 $[MH]^+$, found 253.0865.

6-(4-Hydroxyphenyl)-2*H*-chromen-2-one (37a). The title compound was prepared from **37** (247 mg, 0.98 mmol) in toluene (12 mL), according to method B. The product was purified by flash chromatography (0−1% MeOH in dichloromethane). Yield: 75 mg (32%), brown crystals. ^{1}H NMR (300 MHz, DMSO) δ: 9.60 (s, 1H), 8.10 (d, J = 9.6 Hz, 1H), 7.94 (d, J = 1.9 Hz, 1H), 7.82 (dd, J = 8.6 Hz, 2.1 Hz, 1H), 7.54 (d, J = 8.5 Hz, 2H), 7.43 (d, J = 8.6 Hz, 1H), 6.88 (d, J = 8.5 Hz, 2H), 6.52 (d, J = 9.5 Hz, 1H) ppm. HRMS (ESI) calculated for $C_{15}H_{11}O_3$ $[MH]^+$ 239.0708, found 281.0702.

6-(4-Chlorophenyl)-2*H*-chromen-2-one (38). The title compound was prepared from **34** (100 mg, 0.44 mmol), *p*-chlorophenylboronic acid (111 mg, 0.71 mmol), potassium carbonate (153 mg, 1.11 mmol), and tetrakis(triphenylphosphine) palladium (4.5 mg, 5 μmol) in 5 mL toluene, according to method E. The product was purified by flash chromatography (ethyl acetate in hexane: 0−10%). Yield: 85 mg (74%), white crystals. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.71−7.79 (m, 2H), 7.65 (d, J = 2.2 Hz, 1H), 7.42−7.55 (m, 5H), 6.50 (d, J = 9.5 Hz, 1H). HRMS (ESI) calculated for $C_{15}H_{10}O_2Cl$ 257.0369 $[MH]^+$, found 253.0377.

Biological Methods. [2,4,6,7-^{3}H]-E1 and [2,4,6,7-^{3}H]-E2 were obtained from Perkin-Elmer, Boston (USA). Other chemicals were purchased from Sigma, Merck, or Roth.

Preparation of 17β-HSD1 and 17β-HSD2 Enzymes for Activity Assays. Recombinant His-tagged human 17β-HSD1 was overexpressed in the BL21-CodonPlus (DE3)-RIL strain of *E. coli* containing the pQE30−17beta-HSD type 1 construct (prepared at the Institute of Experimental Genetics, Neuherberg, Germany). The bacteria were resuspended in PBS and sonicated; the resultant cell homogenate was used as the source of the recombinant enzyme, and it was stored frozen until determination of enzymatic activity.

Recombinant N-29 truncated 17β-HSD2 was overexpressed in JM-107 *E. coli* containing the pGEXII-N-29 17beta-HSD type 2 construct (prepared at the Institute of Experimental Genetics, Neuherberg, Germany). The bacteria were resuspended in PBS and sonicated; the resultant cell homogenate was used as the source of the recombinant enzyme, and it was stored frozen until determination of enzymatic activity.

Inhibition of 17β-HSD1. The synthesized coumarins were tested for their inhibition of 17β-HSD1, in terms of their percentages of inhibition of E1 reduction, using *E. coli* cell homogenates.[49] For **3**, **4**, **5**, **6**, and **6a**, the IC_{50} was also determined. Bacterial homogenate was incubated in 100 mM sodium phosphate buffer, pH 6.5, with E1 and [2,4,6,7-^{3}H]-E1 (final concentrations, 55 nM, 0.6 μCi) at 37 °C, in the absence and presence of potential inhibitors at concentrations of 0.6 μM and 6 μM. For determination of IC_{50} values, the concentrations of **3**, **4**, **5**, and **6** ranged from 30 nM to 24 μM and concentrations of **6a** ranged from 10 nM to 6 μM. The concentration of the cofactor NADPH was 100 μM. Inhibitor stock solutions were prepared in DMSO and diluted with acetonitrile prior to use. The final concentration of acetonitrile in the reactions was adjusted to 1%. The reactions were stopped with addition of ethyl acetate after 10 min (the time needed to convert ca. 25% of the substrate in a control assay without inhibitor). The samples were extracted with ethyl acetate, the solvent was evaporated, and the steroids were dissolved in acetonitrile for separation on a reverse-phase HPLC ODS Hypersil (Thermo Scientific) C18 column. Isocratic HPLC runs were performed at 25 °C and a flow rate of 1 mL/min of acetonitrile/water (45:55) as mobile phase. Detection and quantification of the radioactive steroids were performed using Quickszint Flow 302 (Zinsser Analytic, Frankfurt, Germany) as scintillation fluid, and a Ramona 2000 radioflow detector (Raytest). The assays were performed in duplicate (RSD was less than 15%) for determination of the percentages of inhibition, and in triplicate (RSD was less than 5%) for determination of the IC_{50} for **3**, **4**, **5**, **6**, and **6a**. The conversion was calculated according to the following formula %conversion = $(\%E_2/(\%E_2 + \%E_1) \times 100$, and the inhibition was calculated according to %inhibition = [(%conversion of control − % conversion of sample)/%conversion of control] × 100.

Inhibition of 17β-HSD2. The 17β-HSD2 inhibition assay was performed as described for 17β-HSD1 in terms of the percentage of inhibition, but with the following modifications: sodium phosphate buffer, pH 7.5, with E2 and [2,4,6,7-^{3}H]-E2 (final concentrations, 300 nM, 0.6 μCi), with the inhibitors present at concentrations of 0.6 μM and 6 μM, or 6 μM and 60 μM, with 100 μM cofactor NAD^+ used and the reactions stopped after 5 min (time needed to convert ca. 20% of substrate in a control assay without inhibitor).

ER Affinity. The binding affinity of selected compounds to ERα and ERβ was determined as described previously,[61] with minor modifications. Recombinant human ERα and ERβ (0.9 nM) were incubated with 9 nM [2,4,6,7-^{3}H]-E2 and the selected inhibitor, for 18 h at 4 °C. The concentration range of the inhibitors in the incubation mixtures was 10^{-9}−10^{-5} M. Nonspecific binding was calculated using 6 μM E2 as the competing ligand. After incubation, the ligand−receptor complex was bound to 50% hydroxyapatite in Tris (pH 7.5) buffer and washed four times. For radiodetection, the liquid scintillation cocktail Emulsifier Safe (Perkin-Elmer) was added, and samples were counted in a 1214 Rackbeta liquid scintillation counter (LKB Wallac). Each compound was tested in triplicate (RSD was less than 10%), and for determination of the RBAs, the inhibitor and E2 concentrations required to displace 50% of the receptor-bound ^{3}H-E2 were determined. The RBA values were calculated according to the following formula: RBA[%] = $(IC_{50}(E_2)/IC_{50}(\text{inhibitor}) \times 100$. The RBA value for E2 was arbitrarly set at 100%.

Molecular Modeling. The computational study was carried out on a workstation with 4 dual-core AMD Opteron processors, 16 GB of RAM, a GeForce 7800 graphics card, and 1.2 TB of hard disk space. For the docking experiments, *FlexX 3.1.2* (BioSolveIT GmbH)[63] installed on the 64-bit Fedora 7 was used. For figure preparation, PyMol[68] was used. We used the crystal structure of 17β-HSD1 with co-crystallized E2 (PDB entry 1A27).[64] The active site was defined as the area within 6.5 Å of the co-crystallized E2. Protonations and OH group orientations of the active-site amino acid residues were manually assigned with FlexX GUI. We validated the system by the redocking of co-crystallized E2. The docking was carried out using the default parameters of the program. For establishment of 3D pharmacophore model, we performed superposition of active compounds with ROCS[69] (OpenEye Scientific Software Inc.). Visualization and detection of pharmacophoric parts was done with LIQUID[70] plugin for PyMol.

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